with these effects, intracellular Ca2+ accumulation in response to depolarizing voltage steps to 0 mV (control: 60.8 ± 3.0 nM, n=7) was significantly reduced by AID (29.3 ± 1.6 nM, n=7, P<0.001) and AID W-A (18.6 ± 3.4 nM, n=7, P<0.001) peptides; again representing increased inhibitory AID W-A peptide effects (P<0.05 vs AID). Under these conditions, both AID and AID W-A peptides also attenuated G-protein modulation of Ca2+ current (as induced by 1 μM somatostatin).
These data are consistent with inhibitory CaV2.2 peptides acting to reduce synaptic transmission due to a direct effect on presynaptic VDCCs. The increased inhibitory effect of the AID W-A peptide may represent a starting point to develop inhibitory agents in aberrant CaV2.2 signalling pathways, such as in nociception.

2198-Plat
Ca2+ Signaling Amplification by Oligomerization of L-Type Cav1.2 Channels
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Ca2+ influx via L-type CaV1.2 channels is essential for multiple physiological processes including gene expression, excitability, and contraction. Amplification of the Ca2+ signals produced by the opening of these channels is a hallmark of many intracellular signaling cascades, including excitation-contraction (EC) coupling in heart. Using optogenetic approaches, we discovered that CaV1.2 channels form clusters of varied sizes in ventricular myocytes and that physical interaction between these channels via their C-tails renders them capable of coordinating their gating thereby amplifying Ca2+ influx. Light-induced fusion of wild type (WT) CaV1.2 channels with channels carrying a gain-of-function mutation that causes arrhythmias and autism in humans with Timothy syndrome (CaV1.2-TS) increased Ca2+ currents, diastolic and systolic Ca2+ levels, contractility, and the frequency of arrhythmogenic Ca2+ fluctuations in ventricular myocytes. Our data indicate that these changes Ca2+ signaling resulted from CaV1.2-TS increasing the activity of adjoining WT CaV1.2 channels via protein-to-protein interactions. Our data support the novel concept that oligomerization of CaV1.2 channels can control the amplification of Ca2+ influx in excitable cells.

Platform: Interfacial Protein-Lipid Interactions II

2199-Plat
Synaptotagmin C2 Domain Membrane Targeting: Kinetic and Mechanistic Diversity Among Isoforms from Different Cell Types
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Synaptotagmin (Syt) triggers Ca^{2+}-dependent membrane fusion during secretion via its tandem C2 domains, termed C2A and C2B. The seventeen known human isoforms are active in different secretory cell types, including neurons (SytI and others) and pancreatic β cells (SytVII and others). Here, quantitative fluorescence measurements reveal notable differences in the membrane docking affinities, kinetics, and molecular driving forces for C2A and C2B domains from SytI and SytVII, using vesicles comprised of physiological target lipid mixtures. In agreement with previous studies, the Ca^{2+} sensitivity of membrane binding is greater for both domains from SytVII than for their counterparts in SytI. We demonstrate that for C2A, this increased sensitivity is due to a stronger SytVIIC2A membrane interaction, which involves substantial contribution from the hydrophobic effect. Association and dissociation rate constants for both SytVII domains are found to be significantly slower than their counterparts in SytI. For SytVIIC2A, the dissociation rate constant is ~50-fold slower than SytIC2A and is reminiscent of the $cPLA_2C2$ domain that is known to insert deeply into membranes. Addition of sodium sulfate decreases the dissociation rate of SytVIIC2A but not SytIC2A, further indicating that hydrophobic contacts play a major role in SytVIIC2A membrane docking. Thus, SytVIIC2A docks to membranes via both hydrophobic and electrostatic interactions, while the membrane docking interaction of SytIC2A is predominantly electrostatic. The inclusion of phosphatidylinositol-4,5-bisphosphate (PIP_2) in membrane mixtures leads to increased affinity and slower dissociation for both C2B domains, but has minimal effects on C2A domains. Overall, highly homologous domains from these two proteins exhibit distinct mechanisms of membrane binding that may reflect their functions in different cell types.

2200-Plat
How PIP2 Lipids Regulate the Position and Phosphorylation of the Syntaxin N-Terminus
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Syntaxin, a member of the family of soluble N-ethylmaleimide-sensitive factor attachment protein receptor proteins can bind and regulate plasma membrane ion channels and neurotransmitter transporters (NSS). Studies of such mechanisms for the dopamine transporter (DAT) have established the N-terminal segment of Syntaxin as the site of direct interactions, and have shown the critical role of highly charged PIP2 lipids in regulating Syntaxin-DAT interactions. We used a computational approach that combines mesoscale continuum modeling of protein-membrane interactions with all-atom molecular dynamics (MD) simulations to compare conformational states of Syntaxin in complex with PIP2-enriched and PIP2-depleted membranes. Our mesoscale approach is based on non-linear Poisson-Boltzmann theory of electrostatics and diffusion-like Cahn-Hilliard dynamics that makes possible the quantitative tracking of lipid-type demixing in the membrane due to the interaction with the protein. The calculations with this method identified strong electrostatic interactions of specific sites of Syntaxin with PIP2 lipids that diffused to their vicinity. MD simulations of the resulting system established that as many as five PIP2 lipid molecules can simultaneously bind Syntaxin. The attending segregation of PIP2 lipids appears to have a dramatic effect on the positioning of the Syntaxin N-terminal segment with respect to the membrane/water interface. These results are discussed in the context of the suggested role of PIP2 lipids in regulating Syntaxin-DAT interactions by modulating phosphorylation of Syntaxin at its N-terminus.

2201-Plat
Structure and Kinetics of PTEN Tumor Suppressor Association with Lipid Membranes
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PTEN is the second most commonly mutated protein in human cancer [1]. The PTEN-PI3K phosphorylation switch regulates cell growth and survival by controlling the levels of $PI(3,4,5)P_3$ in the plasma membrane. PTEN's association with the membrane is critical for bringing its active site in close proximity to the substrate, $PI(3,4,5)P_3$. The crystal structure of a truncated PTEN was determined [2], however, the structure of membrane-bound PTEN remains unknown. In this work, we perform systematic binding studies of PTEN with membranes and report first steps toward a structural characterization of PTEN associated with bilayers.
We compared the binding affinities of wt PTEN, the truncated PTEN [2] and two point mutants, C124S and H93R, to lipid membranes with various anionic lipid (PS, $PI(4,5)P_2$ and $PI(3,4,5)P_3$) compositions using Surface Plasmon Resonance (SPR). PS and $PI(4,5)P_2$ show strong cooperativity in binding to wt PTEN while $PI(4,5)P_2$ and $PI(3,4,5)P_3$ show independent binding to the catalytically inactive C124S mutant. The H93R mutation is spatially separated from the active site as well as the membrane binding motifs, yet results in altered affinities to PS and $PI(4,5)P_2$. The truncated PTEN mutant has an increased affinity to PS-containing membranes over wt PTEN due to an increase in its net positive charge. Neutron reflectivity (NR) experiments were performed to characterize the structure of the PTEN-membrane complex. We observe minimal penetration of the proteins into the lipid headgroup region, indicating that protein association occurs only with the membrane surface. Small, yet significant differences in the NR profiles emphasize the role the point mutations have in altering PTEN's association with the membrane. Molecular dynamics and coarse-grained simulations are currently being performed to interpret structural and orientational details in the NR data.
[1] Nature (2006) 441, 424-430.
[2] Cell (1999) 99, 323-334.

2202-Plat
Exploring Fluorescence Lifetime and Homo-FRET Measurements to Monitor Lysozyme Oligomerization in Anionic Lipid Membranes: Relation to "Amyloid-Like" Fibril Formation
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